Environmental Persistence and Toxicity of Fire-Retardant Chemicals, Fire-Trol® GTS-R and Phos-Chek® D75-R to Fathead Minnows

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EXECUTIVE SUMMARY

Environmental Persistence and Toxicity of Fire-Retardant Chemicals, Fire-Trol® GTS-R and Phos-Chek® D75-R to Fathead Minnows

This study was conducted to provide information on the toxicity and environmental stability of the fire-fighting chemicals, Fire-Trol® GTS-R and Phos-Chek® D75-R to fish. GTS-R and D75-R were environmentally weathered for up to 45 days at mixed retardant concentrations recommended for application from aircraft and ground tankers. The retardants were environmentally weathered for 7, 14, 21 and 45 days on substrates including nonporous polypropylene surfaces, gravel, sand, forest soils of low organic (LO soil, 1.4 percent) and high organic (HO soil, 3.7 percent) matter content. We also tested highly diluted aqueous concentrations that might occur when mixed retardants are flushed into wetlands and ponds by rainwater. These dilute concentrations approximated concentrations that are lethal to 50 percent of exposed fish (LC50 concentration). GTS-R, GTS-R without the corrosion inhibitor YPS (yellow prussiate of soda or sodium ferrocyanide), and YPS alone were tested to determine the importance of the YPS on the persistence of environmental hazard posed by GTS-R. In addition we tested D75-R, a fire-retardant that does not contain YPS. In support of these field studies, laboratory tests were conducted to confirm the toxicity of these chemicals under different irradiance conditions

Persistence of the fire-retardant chemicals was evaluated by observations of mortality induced in fathead minnows (*Pimephales promelas)* during 24 to 96 hour exposures to formulations that had been aged for various periods of time. In addition, ammonia and weak-acid-dissociable (WAD) cyanide concentrations were measured during D75-R, GTS-R, and YPS exposures. (Note: The chemical concentrations listed herein refer to milligrams of dry concentrate per liter of water). The following results were determined:

- In preliminary tests GTS-R and D75-R aged for up to 21 days on non-porous polypropylene surfaces and gravel remained toxic to fish (Figure 1). Thus, rainwater runoff from watersheds treated with recommended mixed retardant concentrations may pose environmental hazard for weeks after application. Cyanide and ammonia were at lethal concentrations in the GTS-R tests and total ammonia concentrations were elevated during the D75-R tests.
- The type of substrate that GTS-R was weathered on (as aerially applied - mixed retardant) significantly affected environmental persistence. A 24-hour exposure to GTS-R weathered for up to 21 days on soils with 3.6 percent organic matter (HO Soil) was not toxic to fish, whereas significant toxicity was induced by GTS-R weathered on soils with 1.4 percent organic matter (LO soil) and sand (Figure 2). Cyanide was at or below detection limits and ammonia occurred at sublethal concentrations during tests with HO soil. Thus, toxicity was not consistently associated with toxic concentrations of cyanide or ammonia. In contrast, GTS-R weathered on sand retained toxic levels of cyanide and ammonia through 21 days of weathering. The absence of YPS reduced the toxicity of GTS-R applied to HO soil.
- A 24-hour exposure of fish to D75-R weathered on soils for up to 45 days was non-lethal, whereas exposure to D75-R weathered on sand induced 25 to 60 percent mortality (Figure 2). As exposure to D75-R continued for 96 hours, mortality increased for all substrate conditions and ranged from 55 percent for HO soil to 100 percent for sand. Ammonia concentrations were within the lethal range only for D75-R weathered on sand

for 7 to 16 days, thus mortality is not consistently associated with ammonia concentration. Cyanide was not detected in any D75-R treatment.

- A 24-hour exposure to dilute aqueous solutions of GTS-R approximating LC50 concentrations was non-toxic to fathead minnows after 7 days of weathering and thereafter (Figure 3). Cyanide and ammonia concentrations in the aqueous GTS-R solution remained below lethal concentrations. These results suggest that the decomposition of GTS-R in aqueous concentrations occurs within 7 days of weathering.
- A 24-hour exposure to dilute aqueous solutions of D75-R that had aged for 7 days was non-toxic after 24 hours of exposure and induced 40 percent mortality after 96 hours of exposure. Ammonia concentrations in aqueous D75-R solutions were within the lethal range after 7 days of weathering but declined to sublethal concentrations thereafter. These results suggest that the decomposition of D75-R occurs after 7 days of weathering.

Figure 1. Percent mortality of fathead minnows exposed for 24 hours to Fire-Trol® GTS-R, Phos-Chek® D75-R, and a control weathered under environmental conditions on non-porous polypropylene and river gravel substrates.

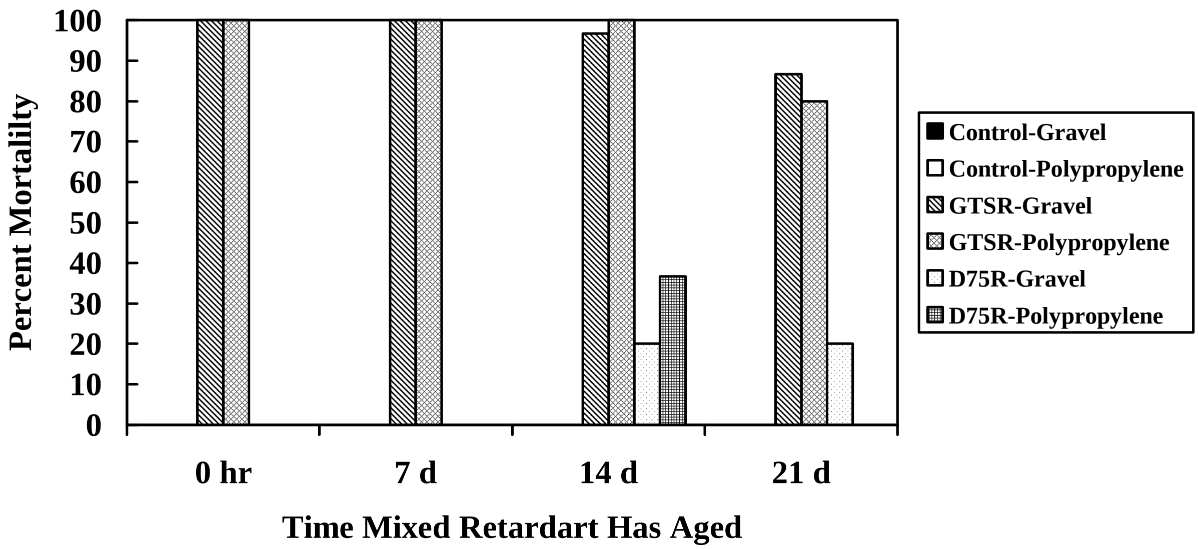


Figure 2. Percent mortality of fathead minnows exposed for 24 hours to Fire-Trol® GTS-R, GTS-R without YPS, YPS alone, Phos-Chek® D75-R, and a control weathered under environmental conditions on non-porous high (HO) and low (LO) organic soils and sand.

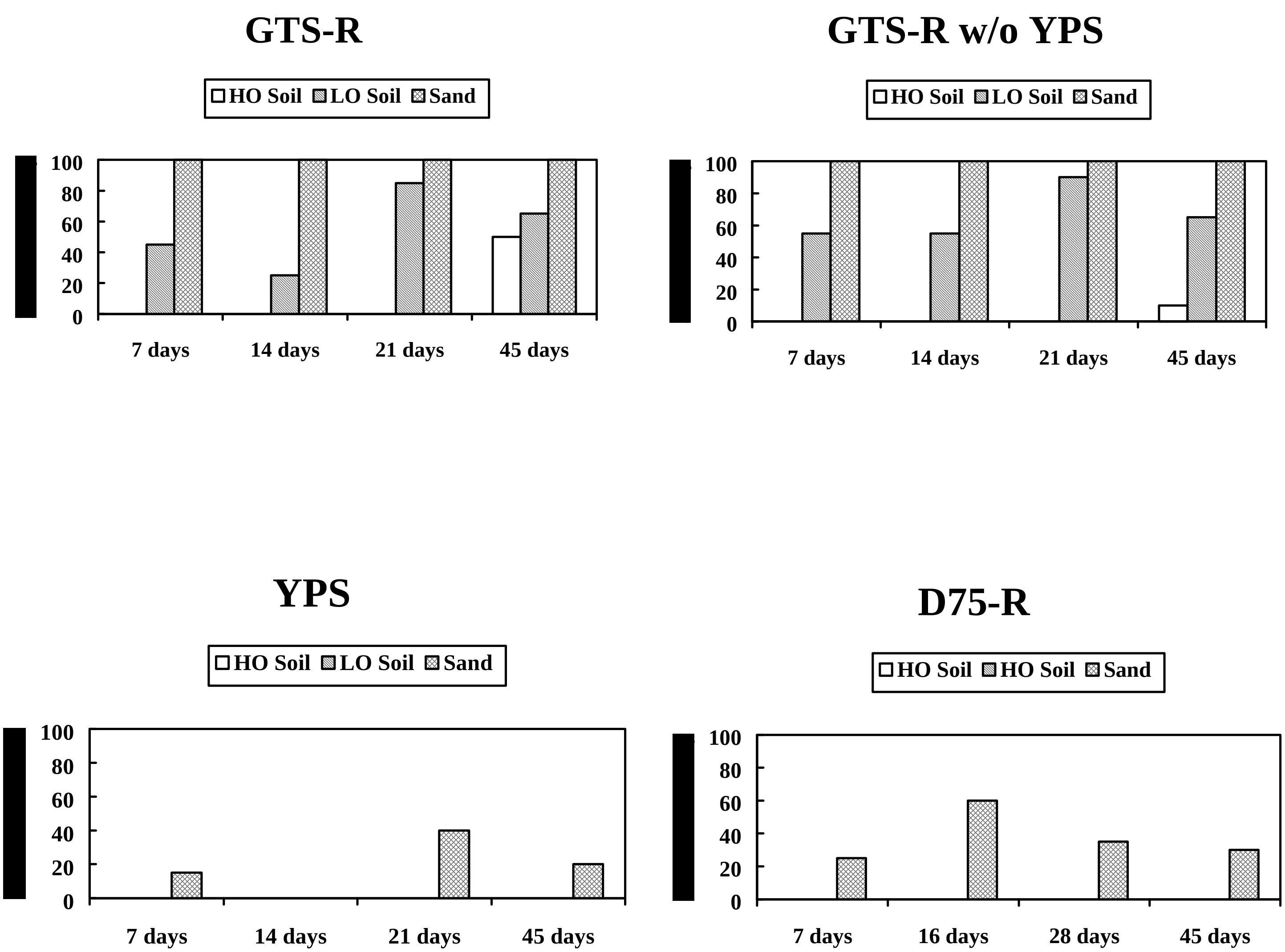
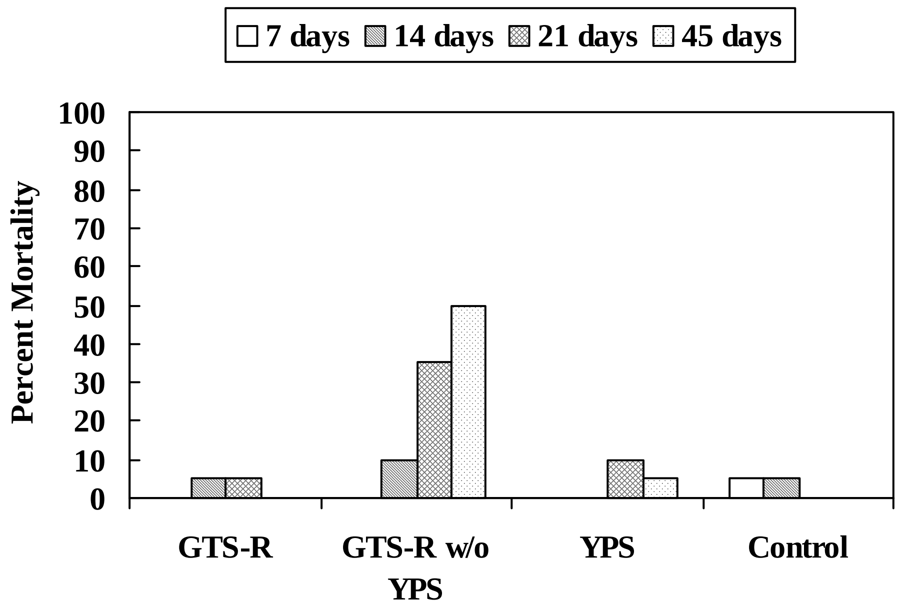


Figure 3. Average percent mortality of fathead minnows exposed for 24 hours to a dilute aqueous solution of Fire-Trol® GTS-R, GTS-R w/o YPS, and YPS alone that had been environmentally weathered for 7, 14, 21, and 45 days.



INTRODUCTION

Fire-retardant chemicals are heavily used throughout the world to extinguish and suppress wildland fires. In contrast to fire-suppressant foams, long-term ammonium-based fire-retardants are applied in high concentrations to provide a combustion barrier after the aqueous component has evaporated. Ecologically, these fire-retardants produce effects consistent with those that occur following agricultural application of fertilizers (Finger et al. 1997). Toxicity is limited in terrestrial habitats, but potentially hazardous in aquatic systems. Some fire-retardant formulations include sodium ferrocyanide (yellow prussiate of soda, YPS hereafter), a corrosion inhibitor that is transformed by sunlight to free cyanide that is toxic to fish and other aquatic organisms. Contamination of aquatic systems may occur through rainwater runoff even though protocols for the application of fire-retardant chemicals prohibit the release of these materials directly in streams, lakes, and wetlands.

The assessment of the environmental persistence of fire-fighting chemicals associated with aerial treatment of forest fires is important for the protection of aquatic resources and for establishing mitigation priorities and goals. Hazard is the combination of toxicity and exposure. Toxicity is the inherent property of a chemical and exposure is the concentration in the environment. The hazards of a compound having brief environmental persistence would be less than compounds that persist for long periods. Preliminary observations indicated persistent toxicity for up to 96 hours of formulations containing YPS. The objectives of this study were (1) to determine the toxicity of GTS-R and D75-R under various irradiance conditions to fathead minnows; (2) determine the persistence of mixed retardants prepared for aerial applications when weathered on different substrates, as measured by toxicity to fish, and the presence of cyanide and ammonia, and (3) determine the persistence of highly diluted aqueous GTS-R and D75-R solutions diluted to LC50 concentrations (concentrations lethal to 50 percent of test organisms) that might occur in receiving waters from rainwater runoff.

METHODS

Test Organisms

Fathead minnow (*Pimephales promelas* - 60 to 90 days post-hatch) were obtained from Aquatic Biosystems, Inc. (Fort Collins, CO) and maintained by the fish culture section of the Columbia Environmental Research Center (CERC). Fish were acclimated to 17 ^{o}C, and held in flow through aquaria (37.5 Liter) supplied with flowing aerated deep well water (pH 7.8, hardness 283 mg/L as $CaCO_3$) for 1 week prior to testing.

Chemical Receipt and Handling

Chemicals selected for testing include Fire-Trol® GTS-R, Fire-Trol® GTS-R without YPS (Fire-Trol Holdings, LLC), Phos-Chek® D75-R (Astaris, LLC), and YPS. The fire-retardant formulations were received as dry concentrates. CERC received test GTS-R, D75-R and YPS on April 28, 2001, and GTS-R without YPS on May 18, 1998 from the U.S. Forest Service Wildland Fire Chemical Systems (WFCS) Program of the Missoula Technology and Development Center, Missoula, Montana via over night courier in sealed 19-liter plastic containers. Upon receipt the shipping container was inspected for damage and the security seals were inspected for evidence of tampering. The chemicals were held in the CERC chemical storage facility at room temperature in the absence of light.

All concentrations, dilutions, and mixtures are based on dry concentrates mixed in water, unless otherwise noted. Mixed retardants used for aerial application were prepared by mixing 192 grams dry concentrate GTS-R/liter of water or 144 grams dry concentrate D75-R per liter of water.   Dry concentrates were also mixed with well water to prepare dilute aqueous solutions (6 mg/liter GTS-R, 47 mg/liter GTS-R without YPS, 0.2 mg/liter YPS, and 170 mg/liter D75-R) approximating the LC50 concentrations.

Experimental Design and Test Conditions

Determination of laboratory LC50s to the fathead minnow. Laboratory tests were conducted to determine the D75-R and GTS-R LC50 concentrations over a 96-hour exposure (LC50). The toxicity testing procedures were conducted in basic accordance with American Society for Testing and Materials Guideline E729, "Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians" (1998). Fathead minnows were exposed in 96-hour static acute toxicity tests to five well water dilutions of fire-retardant chemical and a well water control treatment (pH 7.8, hardness 286 milligrams/liter [mg/liter] as calcium carbonate, alkalinity 258 mg/liter as calcium carbonate).   Exposure to each treatment and control was performed under three different light treatments: dark control (0 micro Watts/square centimeter - $\mu W/cm^2$), light control (0.002 $\mu W/cm^2$ UVB), and UV (4 $\mu W/cm^2$ UVB). Two replicates of each chemical dilution/light treatment were tested.

Toxicity tests were conducted in a solar simulator enclosed with reflective specular aluminum and suspended over a temperature-controlled water bath of similar dimensions (Little and Fabacher 1996). Ten fathead minnows were exposed in 4-liter glass beakers containing 3.5 liters of the chemical solution. Well water was used to prepare a stock solution of each chemical. Prior to placing test organisms in the exposure beakers appropriate volumes of the stock solution were pipetted into the test vessels to obtain the desired exposure concentrations.

Solutions were then mixed thoroughly with a glass-stirring rod. Test beakers containing the organisms were then randomly positioned in a temperature-controlled (17^{o}) water bath under the solar simulator. Test vessels were loosely covered with the appropriate light filters to obtain the desired light treatments as stated above.

Temperature, pH, and dissolved oxygen were measured daily during the test. Cyanide, nitrate and ammonia were measured at the conclusion of the toxicity test. Mortality, condition, and behavior abnormalities such as loss of equilibrium were recorded daily.

Field persistence tests of mixed retardant as applied on fires*:* Preliminary field tests of the persistence of mixed fire-retardant chemicals were conducted under full sun conditions using substrates including the relatively nonporous polypropylene surface of the bucket and 2 cm diameter river gravel. In subsequent tests, sand, forest soil with an organic matter content of 3.6 percent (HO soil), and a 50 percent mixture of forest soil and sand having 1.4 percent organic matter content (LO soil) were used as substrates. The forest soil was collected at the Mark Twain National Forest near Columbia, Missouri. The soil was air dried and sifted to remove debris and create uniform particle size. These soils were characterized by the University of Missouri Agriculture Extension Soil Testing Services (Table 1). One liter of one substrate was placed on the bottom of a 19-liter polypropylene container to provide approximately 3 cm depth of substrate. Mixed retardant was prepared by combining: 199 grams of dry concentrate GTS-R, or GTS-R without YPS to one liter of well water; 145 grams dry concentrate D75-R to one liter of well water; or 1.99 grams of YPS alone to one liter of well water. The YPS treatment approximated the proportion of this chemical (about 1 percent) that is estimated to occur in full formulation GTS-R. We applied 37.8 ml of the mixed retardants and YPS to the surface of substrates to achieve recommended application rate of one gallon per 100 square feet (GPC). This is equivalent to applications of 753 grams GTS-R and 549 grams D75-R per 100 square feet. YPS and GTS-R without YPS were not tested during preliminary assessments using polypropylene or gravel substrates. There was a separate group of exposure containers for each period of weathering duration. These treatments were placed out-of-doors in an unshaded area at CERC where they could be exposed to sunlight, ambient temperature and humidity, but were covered at night. There was no rainfall during these experiments, so no further dilution of the chemical occurred during the outdoor weathering period.

Preliminary toxicity tests were conducted with gravel and polypropylene (container bottom) substrates following each of 7, 14, and 21 days of weathering (Table 2). Subsequent toxicity tests of GTS-R were conducted on sand and soil substrates following 7, 14, 21, and 45 days of weathering (Table 3). Tests of D75-R were conducted following 7, 16, 28, and 45 days of weathering. At the onset of tests, 10 liters of well water were added to the buckets containing the treated substrates. Sediments were allowed to settle for one hour, then 10 fathead minnows were added. The preliminary toxicity tests were conducted for 24 hours with polypropylene and gravel substrates. Later tests with soils and sand were conducted for 96 hours. Temperature, pH, and dissolved oxygen were measured in each exposure chamber daily during the test. Dead fish were removed daily. Cyanide and ammonia were measured at the beginning of each toxicity test.

Field persistence tests of dilute aqueous solutions*:* Aqueous solutions of full formulation GTS-R, GTS-R without YPS, YPS, and D75-R were prepared at the LC50 concentrations and added to 19-liter polypropylene containers to a volume of 10 liters. No substrate was used during these tests. The containers were placed outdoors in an unshaded location for environmental weathering. Toxicity tests were conducted with these LC50 solutions following 7, 14, 21, and 45 days of environmental weathering of GTS-R and D75-R. A separate group of containers was prepared for each weathering period (Table 4). At the onset of the toxicity tests, ten fathead minnows were added to each container and the fish were exposed for 96 hours. During this time they were observed for mortality and water samples were taken for total ammonia, cyanide, dissolved oxygen, pH, and temperature. Dead fish were removed daily.

UV Measurements

Solar measurements were taken outside at 2:00 pm (Central Standard Time) under clear sky conditions during field persistence tests. Laboratory measurements were taken after the lamps of the solar simulator had burned for 1 hour. An Optronic Laboratories® scanning spectroradiometer was used to measure intensity by wavelength at 1 nanometer (nm) intervals over the UVB (290-320 nm), UVA (320-400 nm), and visible (400-700 nm) wavebands. The radiometer was calibrated with a NIST-traceable tungsten lamp within 2 degrees centigrade of ambient air temperature. Checks were made for wavelength accuracy and intensity at intervals during the UV measurements. Measured UV was at target irradiance levels for the laboratory exposures.

Chemical Analysis

Ammonia was measured as total ammonia (NH_4-N) in each treatment of control, treated aqueous, and substrates. Similar analysis was conducted on treatments applied during tests to determine LC50 values. All samples were measured with an ion analyzer (Technicon®

Instruments Corporation, Tarrytown, NY). Un-ionized ammonia (NH_3) concentrations in each treatment were calculated using the ammonia equilibrium equation described by Emerson et al. (1975). In limited studies nitrite was measured at 0, 24, and 96 hours after GTS-R had been applied to HO soil, LO soil, and sand at mixed retardant concentrations. Nitrite was measured colorimetrically using the APHA 4500 NO_2 method (APHA 1998).

Water samples were analyzed for weak-acid-dissociable (WAD) cyanide because this free-ion form of cyanide is most likely to result from photolytic reactions of YPS to sunlight. These samples were placed in 250-ml polypropylene bottles and preserved with sodium hydroxide, and shipped at 4° C via overnight courier to Severn Trent Laboratories (Arvada, CO) for analysis. The samples were analyzed using the standard 4500-CN-I method (APHA 1989).

Statistical Analysis

A five-way factorial ANOVA was conducted on mortality data to determine if toxicity resulted from the interaction of chemical concentration, UV treatment, duration of exposure, or substrate. Probit analysis was used to calculate LC50 values and 95 percent confidence intervals for each chemical based on test concentrations. The criterion of non-overlapping 95 percent confidence intervals was used to determine significant difference ($p \leq 0.05$) between LC50 values (APHA 1989).

RESULTS

Determination of laboratory LC50s for the fathead minnow

The toxicity of GTS-R more than doubled as the duration of exposure increased from 24 hours to 96 hours and varied with UV levels. The 24-hour LC50 values for fathead minnows exposed to

GTS-R were 32.3 mg/liter for the UV treatment; 84.2 mg/liter for the light control; and greater than 50 mg/liter for the dark control. In contrast, the 96-hour LC50 values were 13.6, 19.6, and 32.2 mg/liter for these respective UV treatments (Table 5). The WAD cyanide concentration was estimated at 50 µg/liter at the 13.6 mg/liter LC50 for the UV treatment.

The toxicity of D75-R also increased over time. The 24-hour LC50 concentrations for D75-R were 554.3 mg/liter for the UV treatment; 365.1mg/L for the light control; and 600.6 mg/liter for the dark control. In contrast, the 96-hour LC50 values were 108.2 mg/liter for the UV treatment; 108.8 mg/L for the light control; and 268.4 mg/liter for the dark control (Table 5).

Field Persistence Tests of Mixed Retardants Weathered on Polypropylene and Gravel

In preliminary studies, 24-hour exposures to GTS-R weathered up to 21 days was lethal to fathead minnows and was not diminished by weathering on either the polypropylene surface or on gravel (Table 6). D75-R toxicity tended to increase with weathering. D75-R weathered up to 7 days was not toxic to fish. However, after 14 days of weathering, mortality among fish exposed to D75-R was 20 to 36 percent (Table 6). D75-R also remained toxic to fish after 21 days of weathering on gravel.

Cyanide concentrations varied with substrate in tests with GTS-R, ranging from 523 µg/liter on the hard surface to 213 µg/liter on gravel at time 0. Cyanide concentration decreased over time on both substrates, declining from 523 µg/liter at time 0 to 180 µg/liter after 21 days of weathering on polypropylene (Table 7). In tests with the gravel substrate, cyanide concentrations in GTS-R were less than 50 percent of the concentrations observed when the mixed retardant was applied on polypropylene. Cyanide declined over time ranging from 213 µg/liter at time 0 to 58 µg/liter after 21 days on gravel, dipping to 9 mg/liter following 14 days of weathering. WAD cyanide was not detected at any time in the control chambers, nor was it detected in the D75-R treatments.

Total ammonia concentrations for GTS-R and D75-R were within lethal range (>21 mg/liter, Fairchild et al. 2000) throughout 21 days of weathering (Table 7). In tests with GTS-R, un-ionized ammonia was within a range of concentrations lethal to fathead minnows (>0.8 mg/liter, Fairchild et al. 2000) after weathering for 7 days. In tests with D75-R, the un-ionized ammonia was below the range lethal to fathead minnows following 7 days of weathering, and thereafter. The ammonia concentrations were somewhat lower on gravel substrates for both fire-retardants. The pH of the fire-retardant exposures ranged from 7.1 to 7.5 on day 7; 7.4 to 8.4 on day 14; and 7.1 to 8.1 on day 21 of weathering.

Field Persistence Tests of Mixed Retardants Weathered on Soils and Sand

Water quality parameters remained within acceptable limits during all tests with GTS-R and D75-R (Tables 8 and 9).

Tests of GTS-R: Persistence of GTS-R toxicity was influenced by the type of substrate it was weathered on. GTS-R was weathered on one square foot of high organic soil (HO soil), low organic soil (LO soil), or sand at the minimum recommended application rate of 1 GPC (7.5 grams in 38 ml well water). A 96-hour static exposure to all substrates treated with GTS-R was 70 to 100 percent lethal to fathead minnows following 7 to 45 days of weathering. However, observations of fathead minnow mortality following 24 hours of exposure to treated substrates indicated that the substrate GTS-R was weathered on influenced its short-term toxicity. A 24-hour exposure to GTS-R that had been weathered on HO soil from 7 to 21 days was not lethal to fathead minnows, but exposure to GTS-R weathered for 45 days on HO soil induced 50 percent mortality (Table 10). Mortality induced by GTS-R weathered on LO soil ranged from 25 to 85 percent and appeared to be independent of the duration of weathering, with the lowest mortality following 14 days of weathering and the greatest mortality following 21 days of weathering (Table 10). GTS-R weathered on sand for up to 45 days was lethal to 100 percent of the fathead minnows (Table 10). In contrast, no mortality occurred among fathead minnows exposed to control conditions during the 24 and 96-hour exposures under any substrate or weathering treatment.

Substrate significantly influenced WAD cyanide concentrations of GTS-R after weathering on soils and sand. WAD cyanide was at or below detection limits after weathering on the HO or LO soil for up to 45 days. Lethal concentrations of WAD cyanide were observed when GTS-R was weathered on sand for 7 (155 μg/liter), 14 (41 μg/liter), 21 (54 μg/liter) and 45 (20 μg/liter) days (Table 11).

Substrate also influenced ammonia concentrations of GTS-R. Total and un-ionized ammonia remained below lethal concentrations after weathering for 7 to 45 days on HO soil (Table 11). Total ammonia in GTS-R weathered on LO soil was greater than that observed in the HO soil and un-ionized ammonia approached lethal concentrations at 7 days of weathering, declining thereafter. Total ammonia concentrations ranged from 44.9 to 98.7 mg/liter in GTS-R weathered for over 45 days on sand. Un-ionized ammonia in GTS-R remained within the lethal range after weathering on sand for 7 to 21 days, but declined to sublethal concentrations following 45 days of weathering (Table 11). In limited studies, nitrite concentrations were observed to increase from non-lethal to lethal concentrations as mortality increased from 0 to 100 percent over the 96-hour exposure to GTS-R applied on HO soils.

Tests of GTS-R without YPS: Observations of fathead minnow mortality following 24 hours of exposure to treated substrates indicate that substrate significantly influenced the short-term toxicity of GTS-R without YPS. GTS-R without YPS was not toxic following 7 to 21 days of weathering on HO soil, but induced 10 percent mortality following 45 days of weathering (Table 10). In contrast, a 7 to 45 day weathering of GTS-R without YPS induced 55 to 90 percent mortality on LO soil and 100 percent mortality on sand. When exposures continued through 96 hours, mortality induced by a 96-hour exposure to GTS-R without YPS weathered on HO soil increased from 15 percent following 7 days of weathering to 40 percent following 45 days of weathering. Exposures to GTS-R without YPS weathered on LO soil and sand induced 100 percent mortality at all weathering periods.

Cyanide was not detected in any treatment of GTS-R tested without YPS. Total and un-ionized ammonia were mainly at sublethal concentrations throughout 45 days of weathering on the HO soil. Lethal concentrations of un-ionized ammonia were observed following 7 and 14 days of weathering on the LO soil and sand (Table 11). Thus, toxicity observed after 14 days of weathering appeared to be unrelated to ammonia concentrations (Table 11).

Tests of YPS: During a 24-hour exposure, YPS weathered on HO soils and LO soils did not induce any fathead minnow mortality. However, when weathered on sand YPS induced 15 percent mortality after 7 days, no mortality after 14 days, 40 percent mortality after 21 days and 20 percent mortality after 45 days of weathering (Table 10). In contrast, the 96-hour exposure to YPS was lethal to 100 percent of fish in all YPS treatments weathered on soil and sand from 7 to 45 days. Cyanide concentrations ranging from 13 to 19 μg/liter resulted from weathering YPS on HO soil and were similar to concentrations observed (14 to 25.5 μg/liter) when this material was aged on low organic soil. In contrast, when YPS was weathered on sand, cyanide concentrations ranged from 56 to 170 μg/liter. Total and un-ionized ammonia were well below lethal concentrations for all treatments throughout the study (Table 11).

***<u>Tests of untreated soils and sand</u>*:** There was no fathead minnow mortality among any of the control treatments at any time during tests with GTS-R, GTS-R without YPS, and YPS (Table 10). No detectable cyanide was observed for any of the control treatments weathered on soils or sand for 7 to 14 days, but sublethal amounts of cyanide ranging from 10.8 to 17.5 μg/liter were observed after 21 and 45 days of weathering on soils and sand. Total and un-ionized ammonia concentrations remained at sublethal concentrations throughout the study (Table 11).

<u>Tests of D75-R:</u> A 24-hour exposure to D75-R weathered on HO and LO soil was not toxic, however D75-R weathered on sand for 7 to 45 days induced fathead minnow mortality ranging from 25 to 60 percent with the greatest mortality occurring after 16 days of weathering (Table 12). No mortality occurred among any of the D75-R control conditions.

In contrast to the short-term exposures, 96-hour exposures induced considerably greater mortality. For D75-R weathered on HO soil for 7 days, toxicity increased from 0 percent at 24 hours to 55 percent at 96 hours of exposure; on LO soil toxicity increased from 0 to 80 percent; and on sand toxicity increased from 25 to 100 percent. Toxicity declined with increasing duration of weathering on LO and HO soils. The lethality of D75-R weathered on HO soil declined from 55 percent following 7 days of weathering to 15 percent after 28 and 45 days of weathering, but mortality remained at 90 to 100 percent for D75-R weathered on sand. No mortality occurred among controls with the exception of 10 to 15 percent mortality after 96 hours of exposure to untreated HO soil.

Cyanide was not detected in any D75-R treatment or controls. In tests with soils, total and un-ionized ammonia remained below lethal concentrations throughout the 45-day weathering period, whereas un-ionized ammonia was present in lethal concentrations following 7 and 16 days of weathering on sand (Table 13). Total and un-ionized ammonia concentrations of controls remained at minimal levels for all substrate conditions.

Field Persistence Tests of Dilute Aqueous Solutions

These tests determined the persistence of toxicity of highly diluted fire-retardant chemicals that might occur in rainwater runoff following application. Water quality parameters were within acceptable limits throughout the studies with weathered solutions of GTS-R and D75-R at LC50 concentrations (Tables 14 and 15).

***Tests of GTS-R*:** LC50 solutions (6 mg/liter) of GTS-R that were weathered out-of-doors from 7 to 45 days were not significantly toxic to fathead minnows during any of the 96 hour exposures (Table 16). WAD cyanide was below detection limits for all treatments over time (Table 17). Thus, the 7 days of weathering was sufficient to degrade dilute aqueous solutions of GTS-R. Total and un-ionized ammonia were below lethal concentrations at all times.

***Tests of GTS-R without YPS*:** The LC50 concentration (47 mg/liter) of GTS-R formulated without YPS was not toxic after 7 and 14 days of weathering, however 35 percent and 50 percent mortality were induced by exposure to solutions that had weathered for 21 days and 45 days, respectively (Table 16). There were no detectable levels of WAD cyanide in any treatment. Total ammonia and un-ionized ammonia were within a sublethal range of toxicity for all treatments, with the exception of tests following 14 days of weathering, when un-ionized ammonia reached 0.64 mg/liter (Table 17).

Tests of YPS: The LC50 concentration (0.2 mg/liter) of weathered YPS was not toxic in solutions that had weathered for 7, 14, or 45 days but induced 20 percent mortality following 21 days of weathering (Table 16). As with the GTS-R formulation with YPS, WAD cyanide appeared to rapidly depurate under ambient environmental conditions. WAD cyanide concentrations were below analytical detection limits for all treatments throughout this test series (Table 17). Total and un-ionized ammonia concentrations remained well below toxic levels for all treatments.

Tests of D75-R**:** A 24-hour exposure to the LC50 concentration (170 mg/liter) of D75-R aged from 7 to 45 days was non-lethal. However a 96-hour exposure induced 40 percent mortality following 7 days of weathering, and 10 percent mortality following 16 days (Table 18). No mortality was induced by exposure to LC50 concentrations of D75-R weathered for longer than 16 days. No mortality was observed among controls. No cyanide was detected at any time in any D75-R treatments or controls, and lethal concentrations of un-ionized ammonia were only observed in the D75-R treatment following 7 days of weathering (Table 19).

DISCUSSION

Responses of fathead minnows exposed to GTS-R under laboratory and field conditions were comparable. The 96-hour LC50 concentrations for fish exposed to GTS-R in the laboratory (13.6 to 32.2 mg/liter) were similar to 96-hour LC50 values determined during a 96-hour pond enclosure studies (21.1 to 70.8 mg/liter) under various UV and sediment treatment conditions (Little and Calfee 2002). The 96-hour LC50 concentrations for D75-R ranged from 108 to 268 mg/liter during laboratory studies, however no significant toxicity was observed during the pond enclosure studies. In laboratory tests, fathead minnow sensitivity was within the range of that observed for rainbow trout juveniles with 96-hour LC50 concentrations ranging from 6 to 34 mg/liter GTS-R and 168 mg/liter D75-R, and was similar to that of the southern leopard frog that ranged from 22 to 78 mg/liter for GTS-R and 269 to 293 mg/liter for D75-R (Little and Calfee 2000). In tests with all species, the lowest LC50 value reflected enhanced toxicity induced by UV photoactivation.

With the exception of recommended application rates, there is, to our knowledge, no information available about environmental concentrations that result from the use of any long-term fire-retardant. Application at minimum recommended rates (1 GPC) may result in 1.6 pounds of GTS-R or 1.2 pounds D75-R per gallon of water spread over a 100 square foot area. Operational protocols specify the avoidance of lakes, streams, and wetland areas during the application of fire-retardant chemicals. However, these materials can potentially enter aquatic systems as diluted rainwater runoff. Assuming minimum recommended application rates of 1 GPC, field applications of GTS-R would need to be diluted by a factor greater than 14,000, and D75-R by a factor greater than 5,000 to achieve sublethal concentrations. Such dilution factors for receiving waters would clearly be relevant to small ponds and first-order streams commonly populated by aquatic lifestages of amphibians and certain fish species.

In addition to application rates and dilution ratios, the environmental toxicity of fire-retardant chemicals will depend on the environmental persistence of the formulation. Decomposition of chemical substances can occur through photolytic destruction by sunlight, by chemical processes that bind or convert the material to reduce availability, or by microbial decomposition processes.

There are several potentially toxic components in the GTS-R and D75-R formulations, including total ammonia, un-ionized ammonia, and also cyanide in GTS-R. The most toxic form of ammonia is un-ionized ammonia (NH_3), a neutrally charged molecule (Emerson et al. 1975) that readily crosses cell membranes of fish gills, one of the primary exchange sites for ammonia as a waste product. If environmental NH_3 is present in high concentrations, ammonia will diffuse back into gill tissue and cause waste products to accumulate to toxic levels in fish. As pH increases, the ratio of un-ionized to ionized ammonia increases (Thurston et al. 1983; Thurston and Russo 1983). In addition, as temperature increases at a given pH, ammonia toxicity also increases (DeGraeve et al. 1987). Fairchild et al. (2000) determined toxicity of total (NH_4^+) and un-ionized ammonia for juvenile fathead minnows tested in CERC well water at 25 ^{o}C. Based on these data, total ammonia (>21mg/liter) and un-ionized ammonia (>0.8 mg/liter) concentrations were within the lethal range for the fathead minnow in tests with GTS-R and D75-R that were weathered on polypropylene and gravel. Ammonia was at lethal concentrations in tests of D75-R and of GTR-R (with and without YPS) weathered on sand and approached lethality when weathered for up to 16 days on LO soil. Although ammonia values were consistently below lethal concentrations when these compounds were weathered on LO and

HO soils for 28 to 45 days, significant lethality was observed.

YPS is added to GTS-R as a corrosion inhibitor. As YPS is weathered by ultraviolet radiation in sunlight (or artificial light), it breaks down into labile cyanide complexes, including weak acid dissociable cyanide (CN^-), a form that is water soluble and toxic to aquatic organisms (Burdick and Lipschuetz 1950). In the present study, the estimated cyanide concentration was 50 μg/liter at the 96-hour LC50 for GTS-R. This corresponds to values of 83 to137 μg/liter cyanide reported for juvenile fathead minnows (Smith et al 1978; 1979). No cyanide was detected during any D75-R exposures.

Cyanide concentrations exceeded lethal levels in GTS-R treatments that had been weathered for up to 21 days on polypropylene, gravel, and sand. The observed cyanide concentrations ranged from 0.4 to 10.5 times the estimated LC50. As with ammonia, the apparent contribution of YPS to the overall toxicity of GTS-R was not consistent. On one hand, GTS-R weathered on sand yielded lethal concentrations of cyanide and was consistently toxic. On the other hand, cyanide was not consistently associated with mortality. Significant mortality resulted from exposure to GTS-R weathered for 21 days and longer on LO soil and for 45 days on HO soils even though cyanide was at or below detection limits for these treatments.

That mortality was not consistently caused by the ammonia or cyanide concentration of the fire-retardants may reflect the chemical changes that occurred during the weathering period. The dramatic increase in toxicity of the fire-retardant compounds that occurred between 24 hours and 96 hours of exposure may reflect several variables acting alone or in combination during the exposure. Limited observations during the present study indicate that nitrite concentrations increased to lethal levels during the 96-hour exposure to GTS-R that had been applied on HO soil. Elevated nitrite concentrations were also observed in previous studies with ammonia-based fire retardants (Gaikowski et al. 1996). The conversion of ammonia to nitrite is a microbial process that could be initiated at the onset of exposure and increased as the microbial population expanded. HO soils were likely enriched with microbes, maximizing the effects. Similar processes would likely also occur in tests with GTS-R without YPS and with D75-R. In natural systems nitrite is rapidly converted to non-toxic nitrate (Russo 1985), so the appearance of nitrite in this study may be an artifact of the static exposure method. The delayed mortality may reflect the longer duration of exposure. This is consistent with the decrease in LC50 concentrations observed between 24 and 96 hours for both chemicals. Because of the high concentrations used during the weathering studies, many chemical constituents may have occurred at toxic levels and contributed to the overall toxicity of the exposure, including compounds other than ammonia and cyanide such as toxic products that resulted from weathering.

In interpreting the outcome of the 24- and 96-hour exposures to weathered fire retardants, we consider the 24-hour data to be the most pertinent to environmental exposures. The 24-hour data reflect toxicity that would occur when weathered fire-retardants are rinsed from the landscape during rainfall. Under such conditions, exposures would likely be of short duration as the receiving waters dilute the pulse of fire-retardant then rinse the fire-retardant downstream. A 96 hour exposure would probably be limited to small impoundments of water with limited rinsing or diluting capacity.

The substrates that fire-retardant chemicals are applied on is very important in the resultant environmental persistence of these compounds. Concentrations of ammonia and cyanide observed from tests with gravel were less than half that observed from tests with polypropylene and considerably less when soils were used. A comparison of toxicity at 24 hours of exposure clearly indicates the importance of substrate quality on the persistence of fire-retardant toxicity. For every chemical tested, toxicity was lowest for materials aged on HO soil, intermediate for materials aged on LO soil and greatest for materials aged on sand.

The organic matter content of the substrate may be an important variable related to the persistence of the toxic components of GTS-R and D75-R. HO soil was a loamy forest soil with an organic matter content of 3.6 percent. LO soil was a mixture of forest soil and sand that had an organic matter content of 1.4 percent, and was less effective in mitigating GTS-R and D75-R persistence. Cyanide and ammonia persisted on sand (0.2 percent organic matter) after weathering for 21 days. Poulton (1997) found the chemical half-life of GTS-R decreased as clay content of soils increased, with the most rapid degradation occurring in silty clay loam with 7.5 percent organic matter content. The cation exchange capacity (CEC) of soil is a quantitative measure of the ability of a soil to sorb and retain positively charged ions (cations) against leaching. Clay and organic matter type and content are the primary constituents responsible for the CEC. Generally, the higher the content of clay and/or organic matter, the greater the CEC (Sumner and Miller 1996).

The results indicate that GTS-R and D75-R are environmentally persistent, thus in sandy or rocky watersheds runoff could be toxic well after the application of the chemical. However, this study also clearly indicates that soil composition is likely to be an important variable in the persistence and long-term hazards of GTS-R and D75-R. In the present study, cyanide was not detectable, ammonia was at sublethal concentrations, and no toxicity resulted from exposure to GTS-R or D75-R that had been aged for 1 week or longer on a loamy forest soil with a carbon content of 3.6 percent.

The weathering studies also indicate that the toxic effects of GTS-R or D75-R heavily diluted by rainwater in watershed runoff is likely to be of limited persistence. Neither GTS-R nor D75-R diluted to LC50 concentrations were toxic following 7 days of solar exposures. These results are consistent with earlier pond enclosure studies with fathead minnows (Little and Calfee, 2002) that failed to detect lethal concentrations of ammonia or cyanide after 96 hours of exposure to GTS-R or D75-R under limited UV conditions. In addition, the pond enclosure studies with GTS-R indicated that the greatest mortality occurred within the first 24 hours of fire-retardant application.

CONCLUSIONS

Results of weathering studies under ambient autumn irradiance conditions indicate that GTS-R and D75-R applied at minimum recommended application concentrations (1 GPC) may persist in the environment for at least 21 days, and possibly longer on rocky or non-porous surfaces. Therefore, delayed toxicological effects could occur well after the application of the chemical. However, GTS-R and D75-R are considerably less persistent and less toxic on soils. Toxicity declined as soil composition increased in clay and organic matter. Thus soil composition appears to be a critical variable when evaluating the environmental hazards of these fire-retardant chemicals. Even though application concentrations are quite high relative to LC50 concentrations for fish, the weathering of these materials on soils having 3 to 5 percent organic matter would rapidly diminish toxicity of short-term exposures. Low concentrations of GTS-R or D75-R resulting from rainwater dilutions are not likely to persist in the environment. When GTS-R and D75-R were diluted to LC50 concentrations, cyanide was not detected, levels of ammonia were minimal, and no significant fathead minnow mortality was observed following 7 days of weathering.

ENVIRONMENTAL IMPLICATIONS

If 5 cm of rain fell on an area treated with 1 GPC of mixed GTS-R, 755 grams would be contained in about 472 liters of water. A worse case scenario assuming complete runoff would result in a dilution of the retardant to a final concentration of 1.6 grams GTS-R/liter of water, an amount 117 times the LC50 for GTS-R for the fathead minnows (13.6 mg/liter) determined in this study. Short-term exposures are anticipated to occur when GTS-R is dissolved and diluted by rainwater. The environmental hazards posed by exposure (less than 24 hour exposures) to GTS-R applied at recommended application concentrations would likely be reduced when applications occur on soils of high organic matter composition. Whereas, toxicity might persist when this retardant is applied on rocky or sandy substrates. Long-term exposures (96 hours or greater) may occur when receiving waters are of limited volume or receive limited flow. Exposures under such conditions are expected to be lethal regardless of the quality of substrate GTS-R is weathered on, even to weathering durations of 45 days. The modifying factors of the receiving waters may further diminish the toxicity of GTS-R, depending on the nature and extent of turbidity and concentration of humic and fulvic acids.

If 5 cm of rain fell on an area treated with 1 GPC of mixed D75-R, 554 grams would be contained in about 472 liters of water. This would result in a concentration of about 1.2 grams/liter, or 12 times the D75-R LC50 concentration for fathead minnows (108 mg/liter) determined in this study. Short-term exposures are anticipated to occur when D75-R is dissolved and diluted by rainwater. The environmental hazards posed by short-term exposure (less than 24 hours) to D75-R applied at recommended application concentrations will likely be reduced when applications occur on soils of high organic matter composition. A longer persistence of toxicity may occur if this retardant is applied on rocky or sandy substrates. Long-term exposures (96 hours or greater) may occur in receiving waters of limited volume or flow. Under these circumstances D75-R concentrations will likely be lethal to fish. However, the toxicity may be further reduced by the turbidity and concentrations of humic or fulvic acids in the receiving waters.

The overall contribution of fire-retardant chemicals to the hazards posed by wildland fires may be inconsequential in view of large amounts of ash that are likely to enter aquatic systems from rainwater runoff and elevated water temperatures that may occur especially in shallow waters. Low concentrations of ash can clog fish gills and interfere with respiration.

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Table 1. Characteristics of soils and sand substrates used for Fire-Trol® GTS-R and Phos-Chek® D75-R weathering tests. Shown are average values with standard deviations in parentheses. N= 3 samples for each soil type.

Sample ID	pHs	N.A.[1] Meq/100g	O.M.[2] percent	Bray-1 P [3] mg/kg	Ca m.e./100g	Mg m.e./100g	K m.e./100g	Moisture percent	Sand percent	Silt percent	Clay percent	Textural Classification	CEC [4]m.e./100g
HOSoil	7.0 (0)	0 (0)	3.6 (0)	16.00 (0)	22.1033 (0.8748)	0.7600 (0.0525)	0.4300 (0.0075)	16.5	35	50	15	Silt Loam	23.3
LOSoil	7.2 (0)	0 (0)	1.4 (0.12)	14.33 (0.58)	10.4142 (0.5577)	0.4050 (0.0265)	0.2208 (0.0201)	12.3	77	15	8	Sandy Loam	11.0
Sand	7.4 (0.06)	0 (0)	0.3 (0.06)	3.67 (0.58)	1.7100 (0.0502)	0.0725 (0.0075)	0.0258 (0.0058)	4.8	100	0	0	Sand	1.8

HOSoil (Forest Soil) was collected adjacent to a freshwater creek in the Mark Twain National Forest located south of Columbia, Missouri in a hardwood forest under deciduous tree canopy.

LOSoil (50/50 by mass) was created by taking equal amounts (mass) of sand and forest soil with moisture contents as received and listed in the table.

Sand (River Sand) was purchased from ACE hardware (Westlake) in Columbia, Missouri labeled as filter sand. The sand was cleaned and dried prior to analysis.

[1] Neutralizable acidity
[2] Organic matter
[3] Available phosphate
[4] Cation exchange capacity

Table 2. Experimental design and test procedure sequence for weathering tests of Fire-Trol® GTS-R and Phos-Chek® D75-R at mixed retardant concentration on nonporous polypropylene surface and river gravel. Controls were untreated substrates. N=2 replicates with 10 fish per replicate. Following each interval of weathering, 10 liters of water and fish were exposed for 24 hours.

Treatment	Substrate	Age Duration			
		Day 0	**Day 7**	**Day 14**	**Day 21**
GTS-R or D75-R	Polypropylene	2	2	2	2
	River gravel	2	2	2	2
Control (untreated substrate)	Polypropylene	2	2	2	2
	River Gravel	2	2	2	2

Test Procedure Sequence for tests with weathered mixed retardant:

Gravel substrate added to 19-liter polypropylene containers

GTS-R applied to gravel substrate and to containers without gravel (polypropylene substrate)

Control containers with and without gravel received no retardant treatment

Control, GTS-R, and D75-R containers weathered out-of-doors as separate groups for each of the 0, 7, 14, and 21 days duration of weathering for a total of 16 replicated treatments in 48 exposure containers (3 chemical treatments x 2 substrates x 4 weathering treatments x 2 replicates)

Following each interval of weathering 10 liters of water were added to each container

10 fathead minnows added to each container (3 chemicals x 2 substrates x 1 weathering period x 2 replicates = 12 exposures)

Fish exposure conducted for 24 hours

Table 3. Experimental design and test procedure for weathering tests of Fire-Trol® GTS-R, GTS-R without YPS, YPS and Phos-Chek® D75-R at mixed retardant concentration on high organic (HO) soil, low organic soil (LO), and sand. Controls were untreated substrates.

		Age Duration				
Treatment	**Substrate**	**Day 0**	**Day 7**	**Day 14/16**	**Day 21/28**	**Day 45**
GTS-R, GTS-R withoutYPS, YPS, or D75-R	HO Soil	2	2	2	2	2
	LO Soil	2	2	2	2	2
	Sand	2	2	2	2	2
Control (untreated substrate)	HO Soil	2	2	2	2	2
	LO Soil	2	2	2	2	2
	Sand	2	2	2	2	2

HO soil, LO soil, sand added to 19-liter polypropylene containers.

GTS-R, GTS-R without YPS, YPS, and D75-R mixed with water to create recommended mixed retardant application concentration.

Mixed retardant concentration for each chemical applied to each soil and sand substrate at recommended mixed retardant application concentration

Similar volume of untreated water added to each substrate to serve as control treatments

Control and GTS-R containers weathered out-of-doors as separate groups for each of the 7, 14, 21 and 45-day weathering treatments for a total of 48 replicated treatments in 96 exposure containers (4 chemical x 3 substrates x 4 weathering treatments x 2 replicates)

Control and D75-R containers weathered out-of doors as separate groups for each of the 7, 16, 28 and 45-day weathering treatments for a total of 48 replicated treatments in 96 exposure containers (2 chemical x 3 substrates x 4 weathering treatments x 2 replicates)

Following each interval of weathering 10 liters of water added to each container

10 fathead minnows added to each container (4 chemical x 3 substrates x 1 weathering period x 2 replicates = 24 exposures for GTS-R tests) for 96 hour exposure.

10 fathead minnows added to each container (2 chemical x 3 substrates x 1 weathering period x 2 replicates = 12 exposures for D75-R tests) for 96 hour exposure.

Table 4. Experimental design and test procedure sequence for the outside aging tests of dilute aqueous solutions of Fire-Trol® GTS-R full formulation, GTS-R without YPS, technical grade YPS and Phos-Chek® D75-R. N=2 replicates with 10 fish per replicate.

	Age Duration			
Chemical	**Day 7**	**Day 14**	**Day 21**	**Day 45**
GTS-R Full formulation	2	2	2	2
GTS-R w/o YPS	2	2	2	2
D75-R	2	2	2	2
YPS	2	2	2	2
Control (Untreated)	2	2	2	2

Test Procedure Sequence for Tests with Weathered Dilute Aqueous Solutions:

10 liters of well water added to 19-liter polypropylene containers

GTS-R full formulation, GTS-R without YPS, YPS, or D75-R added to water to the LC50 concentration for each chemical.

Similar volume of untreated water added to each substrate to serve as control treatments

Containers with control and chemicals weathered out-of-doors as separate groups for each of the 7, 14, 21 and 45-day weathering treatments for a total of 16 replicated treatments in 32 exposure containers (4 chemical treatments x 4 weathering treatments x 2 replicates)

Following each interval of weathering 10 fathead minnows added to each container (4 chemical x 1 weathering period x 2 replicates = 8 exposures)

Fish exposure conducted for 96 hours

Table 5. Mean concentrations of Fire-Trol® GTS-R and Phos-Chek® D75-R lethal to 50 percent of exposed fathead minnows during 24 and 96 hour exposures under ultraviolet light, light control and dark control conditions. N= two replicated exposures. The 95 percent confidence limits are shown in parentheses.

	Irradiance Conditions					
Fire-retardant Chemical	**UV (4 μW/cm2)**		**Light Control**		**Dark Control**	
	24-hour LC50	*96-hour LC50*	*24-hour LC50*	*96-hour LC50*	*24 hour-LC50*	*96-hour LC50*
GTS-R	32.3 mg/liter	13.6 mg/liter	84.2 mg/liter	19.6 mg/liter	> 50 mg/liter	32.2 mg/liter
	(25.9 to 38.6)	(10.3 to 16.7)	(36.1 to 132.3)	(16.3 to 23.0)	--	(25.7 to 38.7)
D75-R	554.3 mg/liter	108.2 mg/liter	365.1 mg/liter	108.8 mg/liter	600.6 mg/liter	268.4 mg/liter
	(442.1 to 666.7)	(83.2 to 133.2)	(282.7 to 447.5)	(84.6 to 133)	(467.2 to 734.1)	(218 to 318.9)

Table 6. Average mortality (standard error in parentheses) induced in fathead minnow exposed for 24 hours to Fire-Trol® GTS-R, Phos-Chek D75-R (mixed retardant concentrations) and controls weathered under environmental conditions on non-porous polypropylene and river gravel substrates. * indicates significant difference from the same substrate control treatment. †indicates significant difference from 0- hour mortality for the same substrate treatment.

Treatment	**Substrate**	**Aged 0-hr**	**Aged 7-d**	**Aged 14-d**	**Aged 21-d**
Control	Polyproplyene	0	0	0	0
Control	Gravel	0	0	0	0
GTS-R	Polypropylene	100^{*}	100^{*}	97.6^{*}	86.7^{*} (1.5)
GTS-R	Gravel	100^{*}	100^{*}	100^{*}	80^{*} (2.7)
D75-R	Polypropylene	0	0	$36.7^{*\dagger}$ (5.5)	0
D75-R	Gravel	0	0	$20^{*\dagger}$ (2.7)	$20^{*\dagger}$ (1.0)

Table 7. Average weak acid dissociable cyanide (WAD) and total ammonia (TA) as NH_4 (un-ionized ammonia concentration shown in parentheses) observed for Fire-Trol® GTS-R or Phos-Chek® D75-R (mixed retardant concentrations) aged for 0 to 21 days on non-porous polypropylene and river gravel substrates. ND indicates that WAD cyanide was not detected within the minimum reporting concentration of 10 µg/liter. NA indicates data not available.

Treatment	**Substrate**	**Aged 0-hr**			**Aged 7-d**			**Aged 14-d**			**Aged 21-d**		
		WAD CN µg/L	**TA NH_4 mg/l**	**pH**	**WAD CN µg/L**	**TA NH_4 mg/l**	**pH**	**WAD CN µg/L**	**TA NH_4 mg/l**	**pH**	**WAD CN µg/L**	**TA NH_4 mg/l**	**pH**
Control	Polyproplyene	ND	NA	NA	ND	0.1 (0.0)	7.5 (0.05)	ND	0.0 (0.00)	8.1 (0.25)	ND	0.3 (0.1)	7.9 (0.15)
Control	Gravel	ND	NA	NA	ND	0.0 (0.0)	7.5 (0.06)	ND	0.1 (0.00)	8.1 (0.06)	ND	0.1 (0.0)	7.6 (0.06)
GTS-R	Polypropylene	523	NA	NA	373	78.7 (0.8)	7.3 (0)	223	89.8 (0.3)	8.2 (0.40)	180	86.3 (0.03)	7.2 (0.11)
GTS-R	Gravel	213	NA	NA	25	74.0 (0.7)	7.4 0)	ND	56.8 (0.3)	7.9 (0.06)	58.3	55.5 (0.3)	7.5 (0.06)
D75-R	Polypropylene	ND	NA	NA	ND	78.5 (0.3)	7.1 (0.06)	ND	79.4 (0.2)	7.6 (0.26)	ND	79.0 (0.2)	7.1 (0)
D75-R	Gravel	ND	NA	NA	ND	44.2 (0.3)	7.4 (0.06)	ND	32.7 (0.1)	7.9 (0.17)	ND	50.4 (0.2)	7.3 (0.06)

Table 8. Average temperature, pH, and dissolved oxygen (D.O.) concentration (standard error in parentheses) measured from different substrates treated with Fire-Trol® GTS-R, GTS-R without YPS, YPS and controls environmentally weathered for 7,14, 21,and 45 days.

		Time Chemical Aged											
		7 days			14 days			21 days			45 days		
Chemical	**Substrate**	**Temp ^{0}C**	**pH**	**D.O. (mg/L)**	**Temp ^{0}C**	**pH**	**D.O. (mg/L)**	**Temp ^{0}C**	**pH**	**D.O. (mg/L)**	**Temp ^{0}C**	**pH**	**D.O. (mg/L)**
GTS-R	HO soil	15.8 (3.4)	7.5 (0.1)	7.1 (1.1)	15.1 (3.4)	7.5 (0.1)	6.8 (1.1)	11.8 (1.0)	7.5 (0.1)	8.2 (1.4)	12.0 (1.2)	7.4 (0.1)	8.1 (1.4)
	LO soil	17.2 (0.6)	7.9 (0.0)	8.5 (0.5)	17.7 (0.5)	7.8 (0.0)	8.4 (0.5)	12.0 (1.9)	7.6 (0.0)	9.8 (0.3)	12.3 (1.9)	7.5 (0.1)	9.8 (0.4)
	Sand	18.6 (0.7)	7.8 (0.0)	7.6 (0.4)	19.7 (1.2)	7.8 (0.0)	9.7 (0.1)	12.0 (1.5)	7.6 (0.0)	10.6 (0)	11.4 (2.0)	7.6 (0.0)	10.6 (0)
GTS-R w/o YPS	HO soil	15.6 (3.3)	7.5 (0.1)	7.3 (1.0)	14.5 (3.6)	7.5 (0.1)	6.7 (1.2)	12.1 (1.4)	7.4 (0.1)	8.1 (1.5)	11.9 (1.2)	7.4 (0.1)	8.3 (1.3)
	LO soil	17.4 (1.0)	7.9 (0.1)	8.7 (0.5)	17.6 (0.8)	7.8 (0.1)	8.4 (0.5)	12.3 (2.3)	7.6 (0.1)	9.5 (0.6)	11.9 (1.3)	7.5 (0.0)	9.5 (0.3)
	Sand	18.6 (1.0)	7.9 (0.1)	9.7 (0.1)	18.5 (0.2)	7.9 (0.0)	10.0 (0.1)	11.6 (2.1)	7.7 (0.1)	10.8 (0.1)	11.9 (1.6)	7.6 (0.0)	10.8 (0.1)
YPS	HO soil	18.1 (0.6)	7.6 (0.0)	7.9 (0.8)	16.3 (3.3)	7.5 (0.1)	7.6 (0.7)	11.9 (1.2)	7.4 (0.1)	8.5 (1.2)	11.9 (1.3)	7.5 (0.1)	8.7 (1.0)
	LO soil	17.5 (0.8)	7.9 (0.1)	8.7 (0.2)	17.8 (0.7)	7.9 (0.1)	8.6 (0.4)	12.2 (1.9)	7.7 (0.1)	9.9 (0.4)	11.6 (1.5)	7.7 (0.1)	9.5 (0.9)
	Sand	19.6 (0.5)	7.9 (0.0)	9.6 (0.3)	16.7 (4.3)	7.9 (0.1)	9.7 (0.3)	12.2 (1.7)	7.8 (0.1)	11.0 (0.4)	12.6 (1.6)	7.7 (0.2)	10.5 (0.6)
Control	HO soil	14.7 (3.8)	7.4 (0.1)	7.5 (0.8)	14.8 (3.7)	7.4 (0.1)	7.1 (0.9)	12.0 (1.4)	7.4 (0.1)	8.0 (1.3)	12.1 (1.4)	7.6 (0.1)	7.8 (1.2)
	LO soil	14.1 (3.7)	7.7 (0.1)	9.0 (0.3)	14.4 (3.5)	7.7 (0.0)	8.4 (0.5)	11.8 (1.6)	7.8 (0.2)	9.4 (0.4)	11.9 (1.7)	7.9 (0.1)	8.7 (0.6)
	Sand	14.7 (4.3)	7.8 (0.1)	10.9 (0.9)	15.3 (4.4)	7.9 (0.1)	9.7 (0.6)	12.1 (1.4)	8.0 (0.2)	10.5 (0.3)	11.8 (1.2)	8.2 (0.1)	10.6 (0.4)

Table 9. Average temperature, pH, and dissolved oxygen (D.O.) concentration (standard error in parentheses) measured from different substrates treated with Phos-Chek® D75-R and environmentally weathered for 7,16, 28,and 45 days.

		Time Chemical Aged											
		7 days			14 days			28 days			45 days		
	Substrate	**Temp ^0C**	**pH**	**D.O. (mg/L)**	**Temp ^0C**	**pH**	**D.O. (mg/L)**	**Temp ^0C**	**pH**	**D.O. (mg/L)**	**Temp ^0C**	**pH**	**D.O. (mg/L)**
D75-R	HO soil	26.0 (0.0)	7.6 (0.1)	4.6 (0.8)	21.5 (0.6)	7.2 (0.0)	4.5 (0.2)	11.7 (0.6)	7.3 (0.0)	9.3 (0.1)	16.2 (0.0)	7.1 (0.1)	8.8 (0.0)
	LO soil	26.0 (0.0)	7.7 (0.0)	4.9 (0.2)	21.7 (0.6)	7.5 (0.1)	5.8 (0.2)	11.2 (1.1)	7.5 (0.1)	9.3 (0.3)	16.2 (0.1)	7.3 (0.1)	9.5 (0.5)
	Sand	25.8 (0.1)	7.7 (0.0)	6.5 (0.1)	22.1 (0.4)	7.6 (0.3)	8.8 (2.3)	11.1 (0.9)	7.4 (0.0)	10.2 (0.3)	16.1 (0.0)	7.3 (0.1)	10.0 (0.0)
Control	HO soil	25.8 (0.1)	7.4 (0.0)	5.1 (0.1)	22.0 (0.8)	7.5 (0.0)	4.9 (0.1)	12.7 (0.3)	8.5 (0.3)	8.5 (0.3)	16.4 (0.1)	7.1 (0.1)	8.3 (0.3)
	LO soil	25.7 (0.1)	7.7 (0.0)	6.3 (0.6)	22.6 (0.1)	7.6 (0.0)	6.1 (0.0)	11.9 (0.9)	9.1 (0.6)	9.8 (0.3)	16.3 (0.1)	7.4 (0.1)	9.3 (0.7)
	Sand	25.7 (0.1)	7.9 (0.0)	8.6 (2.3)	21.6 (1.0)	7.8 (0.1)	8.2 (0.1)	11.7 (0.6)	10.1 (0.1)	10.1 (0.1)	16.3 (0.2)	7.6 (0.0)	9.9 (0.2)

Table 10. Cumulative mortality (standard deviation in parentheses) among fathead minnows exposed for 24 to 96 hours to Fire-Trol® GTS-R full formulation, GTS-R w/o YPS, YPS, and controls that had been environmentally weathered on different substrates for 7, 14, 21, and 45 days.

Chemical	Substrate	Duration of Environmental Weathering							
		7 days		14 days		21 days		45 days	
		24 hr	96 hr	24 hr	96 hr	24 hr	96 hr	24 hr	96 hr
GTS-R	HO soil	0	$100^{*\dagger}$	0	$70^{*\dagger}$ (0.4)	0	$90^{*\dagger}$ (0.1)	50^{*} (0.0)	$95^{*\dagger}$ (0.1)
	LO soil	45^{*} (0.1)	$100^{*\dagger}$	25^{*} (0.2)	$100^{*\dagger}$	85^{*} (0.1)	100^{*}	65^{*} (0.1)	$100^{*\dagger}$
	Sand	100^{*}	100^{*}	100^{*}	100^{*}	100^{*}	100^{*}	100^{*}	100^{*}
GTS-R w/o YPS	HO soil	0	$15^{*\dagger}$ (0.0)	0	$20^{*\dagger}$ (0.0)	0	$10^{*\dagger}$ (0.1)	10^{*} (0.1)	$40^{*\dagger}$ (0.3)
	LO soil	55^{*} (0.1)	$100^{*\dagger}$	55^{*} (0.2)	$100^{*\dagger}$	90^{*} (0.1)	100^{*}	65^{*} (0.5)	$100^{*\dagger}$
	Sand	100^{*}	100^{*}	100^{*}	100^{*}	100^{*}	100^{*}	100^{*}	100^{*}
YPS	HO soil	0	$100^{*\dagger}$	0	$100^{*\dagger}$	0	$100^{*\dagger}$	0	$100^{*\dagger}$
	LO soil	0	$100^{*\dagger}$	0	$100^{*\dagger}$	0	$100^{*\dagger}$	0	$100^{*\dagger}$
	Sand	15^{*} (0.1)	$100^{*\dagger}$	0	$100^{*\dagger}$	40^{*} (0.3)	$100^{*\dagger}$	20^{*} (0.3)	$100^{*\dagger}$
Control	HO soil	0	0	0	0	0	0	0	0
	LO soil	0	0	0	0	0	0	0	0
	Sand	0	0	0	0	0	0	0	0

* indicates significant difference from same substrate control treatment

† indicates significant difference from 24 hr mortality for same substrate treatment within same duration time

Table 11. Average concentration (standard error in parentheses) of weak acid dissociable cyanide (WAD) total ammonia (TA) and un-ionized ammonia (UA) measured from different substrates treated with Fire-Trol® GTS-R, GTS-R without YPS, YPS, and controls environmentally weathered for 7,14, 21,and 45 days. ND indicates that WAD cyanide was not detected within the minimum reporting concentration of 10 µg/liter.

		Time Chemical Aged											
		7 days			14 days			21 days			45 days		
Chemical	Substrate	WAD CN (µg/L)	TA NH_4 (mg/L)	UA NH_3 (mg/L)	WAD CN (µg/L)	TA NH_4 (mg/L)	UA NH_3 (mg/L)	WAD CN (µg/L)	TA NH_4 (mg/L)	UA NH_3 (mg/L)	WAD CN (µg/L)	TA NH_4 (mg/L)	UA NH_3 (mg/L)
GTS-R	HO soil	ND	17.6 (2.2)	0.23 (0.03)	ND	20.2 (0.9)	0.26 (0.01)	13 (0)	16.5 (0.8)	0.09 (0.01)	ND	17.7 (1.3)	0.08 (0.004)
	LO soil	ND	34.3 (0.8)	0.75 (0.07)	ND	27.0 (3.1)	0.55 (0.05)	14 (15)	32.1 (6.7)	0.27 (0.10)	ND	28.4 (0.2)	0.17 (0.002)
	Sand	155 (35)	92.2 (2.8)	2.08 (0.07)	41 (7)	54.9 (2.4)	1.06 (0.04)	54 (19)	98.7 (2.0)	0.85 (0.08)	20 (0)	44.9 (7.0)	0.38 (0.11)
GTS-R w/o YPS	HO soil	ND	22.1 (0.1)	0.29 (0.01)	ND	21.0 (5.4)	0.27 (0.08)	ND	20.5 (4.2)	0.09 (0.03)	ND	17.9 (4.9)	0.08 (0.01)
	LO soil	ND	36.5 (4.6)	0.88 (0.06)	ND	34.9 (1.1)	0.69 (0.02)	ND	32.35 (4.2)	0.21 (0.06)	ND	32.9 (0.3)	0.22 (0.04)
	Sand	ND	93.0 (5.3)	2.24 (0.18)	ND	55.4 (2.3)	1.53 (0.04)	ND	57.9 (1.8)	0.55 (0.01)	ND	34.8 (16.7)	0.49 (0.28)
YPS	HO soil	14 (1)	0.3 (0.1)	0.00 (0.00)	13 (1)	0.4 (0.0)	0.01 (0.00)	14 (2.8)	0.3 (0.0)	0.00 (0.00)	19 (2)	0.3 (0.1)	0.00 (0.00)
	LO soil	14 (0)	0.4 (0.0)	0.01 (0.00)	26 (0.7)	0.4 (0.3)	0.01 (0.00)	19 (0.7)	0.4 (0.1)	0.00 (0.00)	24 (4)	0.3 (0.1)	0.00 (0.00)
	Sand	170 (57)	0.1 (0.0))	0.00 (0.001)	56 (40)	0.1 (0.0)	0.00 (0.00)	69 (7)	0.1 (0.0)	0.00 (0.00)	80 (71)	0.1 (0.0)	0.00 (0.00)
Control	HO soil	ND	0.2 (0.0)	0.00 (0.00)	ND	0.2 (0.0)	0.00 (0.00)	11 (3)	0.2 (0.0)	0.00 (0.00)	13 (0)	0.3 (0.1)	0.00 (0.00)
	LO soil	ND	0.4 (0.1)	0.01 (0.00)	ND	0.3 (0.0)	0.01 (0.00)	14 (0.7)	0.3 (0.1)	0.00 (0.00)	15 (7)	0.3 (0.1)	0.00 (0.00)
	Sand	ND	0.1 (0.0)	0.00 (0.00)	ND	0.0 (0.0)	0.00 (0.00)	16 (3.5)	0.1 (0)	0.00 (0.00)	18 (5)	0.1 (0.0)	0.00 (0.00)

Table 12. Cumulative mortality (standard deviation in parentheses) among fathead minnows exposed for 24 and 96 hours to Phos-Chek® D75-R that had been environmentally weathered on different substrates for 7, 16, 28, and 45 days.

Chemical	**Substrate**	**Duration of Environmental Weathering**							
		7 days		**16 days**		**28 days**		**45 days**	
		24 hr	***96 hr***	***24 hr***	***96 hr***	***24 hr***	***96 hr***	***24 hr***	***96 hr***
D75-R	HO soil	0	$55^{*\dagger}$ (0.1)	0	$35^{*\dagger}$ (0.2)	0	$15^{\dagger}$ (0.1)	0	$15^{\dagger}$ (0.2)
	LO soil	0	$80^{*\dagger}$ (0.0)	0	$85^{*\dagger}$ (0.2)	0	$70^{*\dagger}$ (0.1)	0	$35^{*\dagger}$ (0.1)
	Sand	25^{*} (0.2)	$100^{*\dagger}$	60^{*} (0.3)	$100^{*\dagger}$	35^{*} (0.1)	$100^{*\dagger}$	30^{*} (0.1)	$90^{*\dagger}$ (0.0)
Control	HO soil	0	0	0	0	0	10 (0.1)	0	$15^{\dagger}$ (0.2)
	LO soil	0	0	0	0	0	0	0	0
	Sand	0	0	0	0	0	0	0	0

* indicates significant difference from the same substrate control treatment

† indicates significant difference from 24 hr mortality for same substrate treatment

Table 13. Average concentration (standard error in parentheses) of weak acid dissociable cyanide (WAD) total ammonia (TA) and un-ionized ammonia (UA) measured from different substrates treated with Phos-Chek® D75-R and environmentally weathered for 7,16, 28,and 45 days. ND indicates that WAD cyanide was not detected within the minimum reporting concentration of 10 μg/liter.

Chemical	Substrate	Duration of Environmental Weathering											
		7 days			16 days			28 days			45 days		
		WAD CN (μg/L)	TA NH_4 (mg/L)	UA NH_3 (mg/L)	WAD CN (μg/L)	TA NH_4 (mg/L)	UA NH_3 (mg/L)	WAD CN (μg/L)	TA NH_4 (mg/L)	UA NH_3 (mg/L)	WAD CN (μg/L)	TA NH_4 (mg/L)	UA NH_3 (mg/L)
D75-R	HO soil	ND	11.9 (0.5)	0.29 (0.16)	ND	8.7 (0.4)	0.06 (0.01)	ND	7.1 (1.5)	0.03 (0.01)	ND	10.4 (3.0)	0.03 (0.00)
	LO soil	ND	13.4 (0.1)	0.40 (0.00)	ND	13.0 (3.5)	0.16 (0.02)	ND	14.6 (1.7)	0.09 (0.02)	ND	11.8 (2.04	0.06 (0.00)
	Sand	ND	38.0 (3.93	1.12 (0.13)	ND	48.1 (9.2)	1.01 (0.73)	ND	23.6 (0.1)	0.12 (0.01)	ND	33.5 (4.2)	0.18 (0.01)
Control	HO soil	ND	0.1 (0)	0.00 (0.00)	ND	0.3 (0)	0.00 (0.00)	ND	0.2 (0.0)	0.15 (0.04)	ND	0.1 (0.0)	0.01 (0.00)
	LO soil	ND	0.0 (0)	0.00 (0.00)	ND	0.1 (0.0)	0.00 (0.00)	ND	0.1 (0.0)	0.13 (0.09)	ND	0.1 (0.1)	0.00 (0.00)
	Sand	ND	0.0 (0)	0.00 (0.00)	ND	0.1 (0.0)	0.00 (0.00)	ND	0.1 (0.0)	0.05 (0.04)	ND	0.1 (0.0)	0.00 (0.00)

Table 14. Average temperature, pH, and dissolved oxygen (D.O.) concentration (standard error in parentheses) measured in dilute aqueous solutions (LC50 concentrations) of Fire-Trol® GTS-R, GTS-R without YPS, YPS, and controls that were environmentally weathered for 7, 14, 21, and 45 days.

Chemical	**7 days**			**14 days**			**21 days**			**45 days**		
	Temp ^{0}C	**pH**	**D.O. (mg/L)**	**Temp ^{0}C**	**pH**	**D.O. (mg/L)**	**Temp ^{0}C**	**pH**	**D.O. (mg/L)**	**Temp ^{0}C**	**pH**	**D.O. (mg/L)**
GTS-R	27.9 (0.1)	7.7 (0.1)	6.9 (0.1)	20.6 (0.1)	8.5 (0.1)	10.6 (0.3)	19.9 (0.1)	7.9 (0.1)	5.8 (0.5)	24.0 (0.2)	7.7 (0.3)	2.0 0.2
GTS-R w/o YPS	28.0 (0.5)	8.0 (0.0)	6.3 (0.1)	20.6 (0.1)	8.7 (0.1)	10.6 (3.0)	20.4 (0.0)	9.3 (0.0)	12.0 (0.6)	24.0 (0.1)	8.6 (1.3)	2.7 (2.3)
YPS	28.0 (0.6)	7.8 (0.1)	6.4 (0.2)	20.0 (0.0)	8.1 (0.1)	6.2 (0.1)	20.3 (0.2)	7.8 (0.0)	5.0 0.1	24.2 (0.1)	7.4 (0.1)	2.3 (0.3)
Control	28.4 (0.1)	7.8 (0.0)	6.6 (0.1)	19.6 (0.1)	7.9 (0.0)	5.9 (0.3)	19.9 (0.2)	7.5 (0.0)	4.7 (0.4)	23.9 (0.1)	7.4 (0.1)	2.8 (1.3)

Table 15. Average temperature, pH, and dissolved oxygen (D.O.) concentration (standard error in parentheses) measured in dilute aqueous solutions (LC50 concentration) of Phos-Chek® D75-R and controls that were environmentally weathered for 7, 14, 21, and 45 days.

Chemical	Time Chemical Aged											
	7 days			14 days			21 days			45 days		
	Temp ^{0}C	**PH**	**D.O. (mg/L)**	**Temp ^{0}C**	**pH**	**D.O. (mg/L)**	**Temp ^{0}C**	**pH**	**D.O. (mg/L)**	**Temp ^{0}C**	**pH**	**D.O. (mg/L)**
D75-R	25.9 (0.1)	8.7 (0.1)	12.2 (2.8)	22.2 (0.1)	9.1 (0.1)	15.2 (0.2)	10.6 (0.4)	8.9 (0.0)	14.5 (0.1)	16.1 (0.0)	8.3 (0.1)	11.0 (4.2)
Control	26.0 (0.1)	7.9 (0.0)	8.2 (0.2)	22.5 (0.2)	8.1 (0.1)	9.1 (0.4)	11.6 (0.7)	10.4 (0.1)	9.6 (1.1)	16.4 (0.0)	8.0 (0.1)	10.8 (0.4)

Table 16. Cumulative average percent mortality (standard deviation in parentheses) of fathead minnows exposed for 96 hours to dilute aqueous solutions (LC50 concentration) of Fire-Trol® GTS-R, GTS-R without YPS, YPS, and controls that had been environmentally weathered for 7, 14, 21, and 45 days.

Chemical	Weathering Duration	Duration of exposure to weathered solution			
		24 hr	**48 hr**	**72 hr**	**96 hr**
GTS-R	7 days	0	0	0	0
	14 days	5 *(0.1)*	5 *(0.1)*	5 *(0.1)*	5 *(0.1)*
	21 days	5 *(0.1)*	5 *(0.1)*	5 *(0.1)*	5 *(0.1)*
	45 days	0	0	0	0
GTS-R w/o YPS	7 days	0	0	0	0
	14 days	10 *(0.1)*	10 *(0.1)*	10 *(0.1)*	10 *(0.1)*
	21 days	35 *(0.1)*	35 *(0.1)*	35 *(0.1)*	35 *(0.1)*
	45 days	50 *(0.7)*	50 *(0.7)*	50 *(0.7)*	50 *(0.7)*
YPS	7 days	0	0	0	0
	14 days	0	0	0	0
	21 days	10	10	15 *(0.07)*	20 *(0.14)*
	45 days	5 *(0.1)*	5 *(0.1)*	5 *(0.1)*	5 *(0.1)*
Control	7 days	5 *(0.1)*	10 *(0.1)*	10 *(0.1)*	10 *(0.1)*
	14 days	5 *(0.1)*	5 *(0.1)*	5 *(0.1)*	5 *(0.1)*
	21 days	0	0	0	0
	45 days	0	5 *(0.1)*	5 *(0.1)*	5 *(0.1)*

Table 17. Average concentration (standard error in parentheses) of weak acid dissociable (WAD) cyanide, total ammonia (TA), and un-ionized ammonia (UA) measured from dilute aqueous solutions of Fire-Trol ® GTS-R (LC50 concentration) with and without YPS, and YPS following 7, 14, 21, and 45 days of environmental weathering. ND indicates WAD cyanide was not detected within minimum reporting concentrations of 10 µg/liter.

Chemical	Time Chemical Aged											
	7 days			14 days			21 days			45 days		
	WAD CN (µg/L)	TA NH_4 (mg/L)	UA NH_3 (mg/L)	WAD CN (µg/L)	TA NH_4 (mg/L)	UA NH_3 (mg/L)	WAD CN (µg/L)	TA NH_4 (mg/L)	UA NH_3 (mg/L)	WAD CN (µg/L)	TA NH_4 (mg/L)	UA NH_3 (mg/L)
GTS-R	ND	1.0 (0.1)	0.03 (0.00)	ND	0.2 (0.0)	0.02 (0.00)	ND	0.6 (0.0)	0.02 (0.01)	ND	0.0 (0.0)	0.00 (0.00)
GTS-R w/o YPS	ND	7.5 (0.2)	0.49 (0.00)	ND	4.5 (0.1)	0.64 (0.14)	ND	0.2 (0.0)	0.09 (0.00)	ND	0.0 (0.0)	0.00 (0.0)
YPS	ND	0.2 (0.0)	0.01 (0.00)	ND	0.4 (0.0)	0.02 (0.00)	ND	0.1 (0.0)	0.00 (0.00)	ND	0.0 (0.0)	0.00 (0.00)
Control	ND	0.3 (0.1)	0.01 (0.00)	ND	0.3 (0.1)	0.00 (0.00)	ND	0.0 (0.0)	0.00 (0.00)	ND	0.0 (0.0)	0.00 (0.00)

Table 18. Average cumulative percent mortality of fathead minnows exposed for 96 hours to dilute aqueous solutions (LC50 concentration) of Phos-Chek® D75-R and controls that had been environmentally weathered for 7, 16, 28, and 45 days.

Chemical	Weathering Duration	Duration of exposure to weathered solution			
		24 hr	48 hr	72 hr	96 hr
D75-R	7 days	0	0	$15^{*\dagger}$ (0.2)	$40^{*\dagger}$ (0.6)
	16 days	0	$10^{*\dagger}$ (0.1)	$10^{*\dagger}$ (0.1)	$10^{*\dagger}$ (0.1)
	28 days	0	0	0	0
	45 days	0	0	0	0
Control	7 days	0	0	0	0
	16 days	0	0	0	0
	28 days	0	0	0	0
	45 days	0	0	0	0

* indicates significant difference from same substrate control treatment

† indicates significant difference from 24 hr same substrate treatment.

Table 19. Average concentration (standard error in parentheses) of weak acid dissociable (WAD) cyanide, total ammonia (TA), and un-ionized ammonia (UA) measured from dilute aqueous solutions (LC50 concentration) of Phos-Chek® D75-R following 7, 16, 28, and 45 days of environmental weathering. ND indicates that WAD cyanide was not detected within the minimum reporting concentration of 10 μg/liter.

Chemical	Time Chemical Aged											
	7 days			16 days			28 days			45 days		
	WAD CN (μg/L)	**TA NH_4 (mg/L)**	**UA NH_3 (mg/L)**	**WAD CN (μg/L)**	**TA NH_4 (mg/L)**	**UA NH_3 (mg/L)**	**WAD CN (μg/L)**	**TA NH_4 (mg/L)**	**UA NH_3 (mg/L)**	**WAD CN (μg/L)**	**TA NH_4 (mg/L)**	**UA NH_3 (mg/L)**
D75-R	ND	5.5 (1.9)	1.3 (0.1)	ND	0.1 (0.0)	0.03 (0.01)	ND	0.1 (0.0)	0.01 (0.00)	ND	0.1 (0.0)	0.00 (0.00)
Control	ND	0.0 (0.0)	0.0 (0.0)	ND	0.0 (0.0)	0.0 (0.0)	ND	0.0 (0.0)	0.02 (0.02)	ND	0.0 (0.0)	0.01 (0.00)